

Neotropical Monogenoidea. 20. Two New Species of Oviparous Gyrodactylidea (Polyonchoinea) from Loricariid Catfishes (Siluriformes) in Brazil and the Phylogenetic Status of Ooegyrodactylidae Harris, 1983

WALTER A. BOEGER,¹ DELANE C. KRITSKY,² AND ELIZABETH BELMONT-JÉGU³

¹ Departamento de Zoologia, Universidade Federal do Paraná, Caixa Postal 19020, Curitiba, Paraná, and Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brasil,

² College of Health Professions, Box 8090, Idaho State University, Pocatello, Idaho 83209, and

³ Laboratório de Ictioparasitologia, Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas 69000, Brasil

ABSTRACT: Two new species of oviparous gyrodactylideans are described from the external surface of Brazilian loricariid catfishes: *Phanerothecium spinatus* sp. n. from *Hypostomus punctatus* (Valenciennes) from Rio Guandú, State of Rio de Janeiro; and *Hyperopletes malmbergi* gen. et sp. n. from *Rhineloricaria* sp. from Igarapé Candiru, State of Amazonas. The monotypic *Hyperopletes* gen. n. is proposed for species having a seminal vesicle located within the copulatory sac, a second external seminal vesicle, and an eversible copulatory organ armed with spines. Cladistic analysis of the oviparous species, *H. malmbergi* sp. n., *Nothogyrodactylus amazonicus* Kritsky and Boeger, 1991, *N. clavatus* Kritsky and Boeger, 1991, *N. plaesiophallus* Kritsky and Boeger, 1991, *Ooegyrodactylus farlowellae* Harris, 1983, *Phanerothecium caballeri* Kritsky and Thatcher, 1977, *P. harrisi* Kritsky and Boeger, 1991, and *P. spinatus* sp. n., and the viviparous Gyrodactylidae (considered a single monophyletic taxon), indicates that Ooegyrodactylidae Harris, 1983, is paraphyletic. Ooegyrodactylidae is rejected, and the genera *Hyperopletes*, *Nothogyrodactylus*, *Ooegyrodactylus*, and *Phanerothecium* are transferred to the Gyrodactylidae Van Beneden and Hesse, 1863. Synapomorphies supporting the new composition of the Gyrodactylidae are presence of (a) a muscular copulatory organ, (b) spike sensilla in the head organs, (c) an unciliated larva (oncomiracidium absent), (d) a deep bar associated with the deep roots of the anchor pair, (e) separate genital pores, (f) a massive Mehlis' gland, (g) an amorphous cap on the egg filament, and absence of (h) a vagina and (i) eyes in the larva and adult. All oviparous genera received evolutionary support except the paraphyletic *Nothogyrodactylus*.

KEY WORDS: Monogenoidea, Ooegyrodactylidae, Gyrodactylidae, *Hyperopletes* gen. n., *Hyperopletes malmbergi* sp. n., *Phanerothecium spinatus* sp. n., Loricariidae, *Rhineloricaria* sp., *Hypostomus punctatus*, cladistics, phylogeny.

During a survey of ectoparasites of Neotropical freshwater fishes from the States of Amazonas and Rio de Janeiro in Brazil, 2 new species of oviparous Gyrodactylidea were recovered from armored catfishes (Loricariidae). In this article, these species are described, and *Hyperopletes* gen. n. is proposed to accommodate 1 of them.

The Ooegyrodactylidae was proposed by Harris (1983) for oviparous Monogenoidea with close relationships to viviparous Gyrodactylidae. Monophyly of Harris' family was challenged by Kritsky and Boeger (1991) because of the apparent absence of synapomorphic features, and Boeger and Kritsky (1993) tentatively considered the family a junior synonym of the Gyrodactylidae in their phylogenetic analysis of the Monogenoidea. To evaluate the evolutionary status of this family, the phylogenetic relationships of oviparous gyrodactylid-like species and the Gyrodactylidae (sensu stricto) are reconstructed us-

ing methods of phylogenetic systematics. Results of this analysis are presented herein.

Materials and Methods

Rhineloricaria sp. were caught during February 1991 by E.B.J. from the "igarapé" Candiru, a small tributary of the Rio Puraquequara on Highway AM-010 near Manaus, Amazonas, Brazil. *Hypostomus punctatus* (Valenciennes) were captured during January 1991 from the Rio Guandú, Nova Iguaçu County, State of Rio de Janeiro, Brazil, by a professional fisherman. Hosts were placed individually or pooled in a container containing a 1:4,000 formalin solution for removal of helminths according to procedures of Putz and Hoffman (1963). After 1 h, the vial was shaken vigorously, and formalin was added to increase concentration to 5%. Some specimens were mounted unstained in Gray and Wess' medium for study of sclerotized structures. Other specimens were stained with Gomori's trichrome for determination of internal morphology. Illustrations were prepared with the aid of a camera lucida. Measurements, all in micrometers, were made according to procedures of Mizelle and Klucka (1953); the av-

erage is followed by the range and number (n) of structures measured in parentheses. Type specimens were deposited in the helminthological collections of the Instituto Oswaldo Cruz (IOC), Rio de Janeiro; the University of Nebraska State Museum (HWML), Lincoln; and the U.S. National Museum (USNM), Beltsville, Maryland.

Transformation series used in the phylogenetic analysis were defined from the literature and available specimens. Homologous series in which the apomorphic state represents an autapomorphy of a single ingroup taxon were not utilized. In group taxa included all species described in *Hyperopletes* gen. n., *Nothogyrodactylus* Kritsky and Boeger, 1991, *Ooegyrodactylus* Harris, 1983, and *Phanerothecium* Kritsky and Thatcher, 1977; the group of viviparous species traditionally comprising the Gyrodactylidae (sensu stricto) was also included as a single in group taxon. For this analysis, viviparity was considered the synapomorphy supporting monophyly of the latter taxon; we assumed that viviparity developed only once in the evolutionary history of the Gyrodactylidae. The Gyrodactylidae (sensu stricto) is represented in the analysis as the viviparous ancestor; the hypothetical ancestor, developed by outgroup and functional outgroup comparison, represents the contrived ancestor of the ingroup (Table 1).

An initial hypothesis on the evolutionary relationships of in group taxa was constructed using Hennigian argumentation (Hennig, 1966; Wiley, 1981); the topology of the cladogram was then subjected to PAUP (Phylogenetic Analysis Using Parsimony, Version 2.4.1; D. L. Swofford, Illinois Natural History Survey, Champaign) to confirm that it was a most-parsimonious tree. A total of 35 character states comprising 19 transformation series was used in the analysis. Polarization of homologous series was determined by outgroup and functional outgroup analyses (Watrous and Wheeler, 1981; Maddison, et al., 1984). The Anoplodiscidae, Bothitrematidae, and Tetraonchoididae were chosen as outgroups based on a previous reconstruction of the phylogeny of the Monogenoidea by Boeger and Kritsky (1993). The matrix is presented in Table 1.

Results

Taxonomic Account

Monogenoidea Bychowsky, 1937

Polyonchoinea Bychowsky, 1937

Gyrodactylidae Bychowsky, 1937

Gyrodactylidae Van Beneden and Hesse, 1863

Phanerothecium spinatus sp. n.

(Figs. 1–7)

HOST AND LOCALITY: *Hypostomus punctatus* (Valenciennes), Loricariidae; Rio Guandú, Nova Iguaçu, Rio de Janeiro, Brazil.

TYPE SPECIMENS: Holotype, IOC 33051a; 30 paratypes, IOC 33051b–k, USNM 82823, HWML 36346.

DESCRIPTION: Body fusiform, 1,124 (775–1,410; $n = 12$) long; greatest width 175 (125–

215; $n = 13$) near midlength; tegument smooth with 6 bilateral pairs of “sensory” pustules spaced along lateral margin from body midlength to distal peduncle. Bilateral excretory pores at level of anterior pharyngeal bulb. Cephalic lobes well developed; head organs conspicuous. Distal pharyngeal bulb 69 (60–80; $n = 9$) in diameter, proximal pharyngeal bulb 69 (63–85; $n = 9$) in diameter. Intestinal ceca terminating at level of posteriormost vitelline follicle. Testis subspherical, variable in size (depending on age of specimen), becoming inconspicuous in adult worms; proximal seminal vesicle with thick wall; distal seminal vesicle elongate; copulatory sac ovate; spines of copulatory organ numerous, minute. Germarium ovate, 70 (45–90; $n = 8$) long, 75 (58–95; $n = 8$) wide; seminal receptacle subspherical. Uterine pore a transverse slit, with sphincterlike muscle. Uterus delicate, containing a maximum of 10 eggs; egg elongate ovate, 162 (151–180; $n = 6$) long, 41 (34–55; $n = 6$) wide; filament moderately long; filament cap urn-shaped. Dextral pregerminal vitelline follicles absent. Peduncle elongate; haptor 87 (79–97; $n = 9$) long, 95 (86–110; $n = 9$) wide. Anchor 59 (52–65; $n = 16$) long, with elongate superficial root, short angular deep root, slightly curved shaft, recurved point; base 25 (17–33; $n = 14$) wide. Superficial bar 48 (32–60; $n = 13$) long, plate like, with slightly enlarged ends; deep bar rod-shaped, variably bent. Hook with straight diagonal shaft, erect thumb, slender proximally tapered shank; hooklet 6 (4–7; $n = 23$) long, shank 29 (27–34; $n = 23$) long, domus $\frac{1}{4}$ shank length.

REMARKS: *Phanerothecium spinatus* sp. n. differs from other congeneric species by having conspicuous spines on the copulatory organ and lacking a keel on the shank of the hooks. The specific name is from Latin (*spinatus* = spined) and refers to the presence of spines on the copulatory organ.

Hyperopletes gen. n.

DIAGNOSIS: Body divisible into cephalic region, trunk, peduncle, haptor. Tegument thin, smooth. Cephalic lobes 2, terminal; each with spike sensilla, portion of head organ. Cephalic glands unicellular, in 2 bilateral groups posterolateral, dorsal to level of pharynx. Eyes absent. Mouth ventral; pharynx comprising 2 tandem bulbs, proximal bulb glandular, distal bulb muscular with digitiform projections; intestinal ceca 2, lacking diverticula, nonconfluent. Worms oviparous, protandrous. Gonads tandem, in-

Table 1. Character matrix used in the reconstruction of the evolutionary relationships of the Gyrodactylidae (sensu nobis).

Taxon	Homologous series*																		
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
<i>Ooegyrodactylus farlowellae</i>	1	1	0	1	1	1	1	0	1	2	1	1	1	0	0	1	9	9	9
<i>Phanerothecium caballeri</i>	1	1	0	1	1	1	1	0	1	9	1	1	9	9	1	1	9	9	9
<i>P. harrisi</i>	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	9	9	9
<i>P. spinatus</i>	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	9	9	9
<i>Nothogyrodactylus clavatus</i>	1	1	0	1	1	1	0	0	0	0	1	1	1	0	1	1	1	1	1
<i>N. amazonicus</i>	1	1	0	1	1	1	0	0	0	1	1	1	1	0	0	1	0	0	1
<i>N. plaesiophallus</i>	1	1	0	1	1	1	0	0	0	0	1	1	1	0	1	1	0	1	1
<i>Hyperopletes malmbergi</i>	1	1	0	1	1	1	1	1	1	2	1	1	1	0	0	1	9	9	9
Viviparous ancestor	1	1	1	1	1	1	2	1	1	2	2	1	9	0	0	1	9	9	9
Hypothetical ancestor	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0

* Homologous series are presented in an order corresponding to that of the character analysis. 9 = missing data.

tercecal; testis postgermarial. External seminal vesicle subspherical. Copulatory sac well defined, with eversible copulatory organ, internal seminal vesicle; copulatory organ armed with spines. Germarium submedian with internal seminal receptacle; ootype surrounded by large Mehlis' gland; uterus with thin wall; uterine pore dextroventral at level of copulatory sac; vagina absent. Uterus containing a maximum of 1 egg; egg with single proximal filament; egg filament embedded in amorphous cap. Vitellaria comprising numerous large, postgermarial follicles. Haptor ventrally concave, with pair of ventral anchors, superficial bar lacking shield, deep bar connecting to deep roots, 16 gyrodactylid hooks marginal; hooks with extrahamular distribution (Kritsky and Mizelle, 1968). Parasitic on external surface of loriciid fishes.

TYPE SPECIES: *Hyperopletes malmbergi* sp. n.

REMARKS: *Hyperopletes* gen. n. is monotypic. Characters that distinguish this genus from other oviparous gyrodactylids are the combined presence of (a) a spined copulatory organ, (b) a seminal vesicle lying within the copulatory sac, and (c) a second external seminal vesicle. Species of the host family Loricariidae are commonly referred to as the "armored" catfishes; the name of the new genus is from Greek (*hyper* = above + *hopl/o* = armor + *-etes* = one who) and has the intended meaning of "the one who dwells on armor."

***Hyperopletes malmbergi* sp. n.**
(Figs. 8–13)

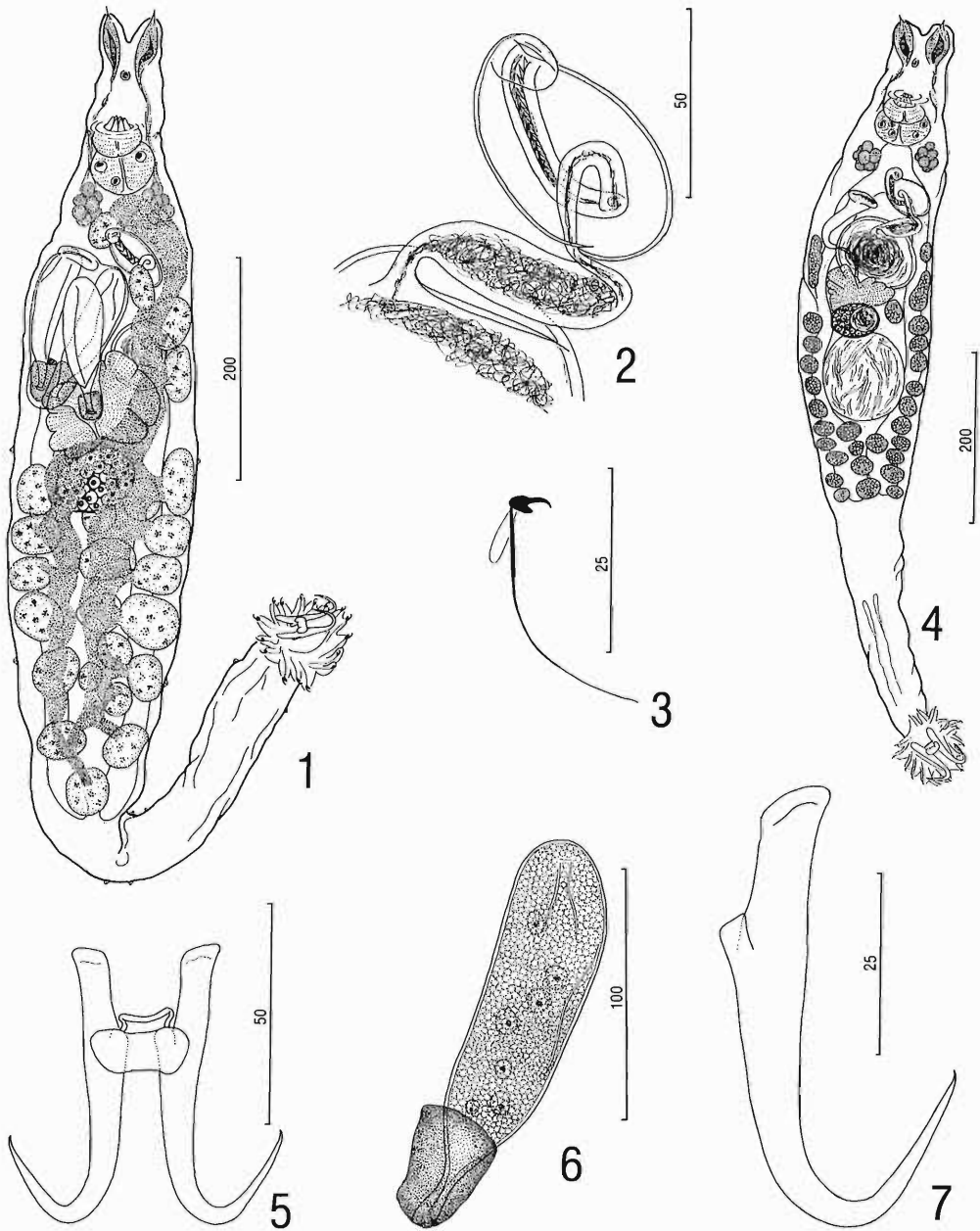
HOST AND LOCALITY: *Rhineloricaria* sp., Loricariidae; Igarapé Candiru, a tributary of Rio Pu-

raquequara, Highway AM-010, near Manaus, Amazonas, Brazil.

TYPE SPECIMENS: Holotype, IOC 33050a; 27 paratypes, IOC 33050b-1, USNM 82822, HWML 36347; 13 vouchers, HWML 36348.

DESCRIPTION: Body robust, fusiform, 682 (460–870; *n* = 13) long; greatest width 143 (105–200; *n* = 15) near midlength. Cephalic lobes well developed; head organs conspicuous. Distal pharyngeal bulb 66 (43–69; *n* = 10) in diameter; proximal pharyngeal bulb 74 (52–95; *n* = 9) in diameter. Testis subspherical, variable in size (depending on age of specimen), becoming inconspicuous in adult worms; proximal seminal vesicle subspherical, delicate; copulatory sac pyriform; copulatory organ armed with 2 regions of numerous small rectangular spines. Germarium ovate, 57 (50–67; *n* = 6) long, 81 (68–102; *n* = 6) wide, with internal seminal receptacle. Uterus delicate; uterine pore surrounded by weak sphincter; egg 145 (132–154; *n* = 4) long, 34 (32–38; *n* = 3) wide, elongate ovate; filament short, flared; filament cap subspherical to subovate. Vitelline follicles extending from level of germarium posteriorly to peduncle. Peduncle short; haptor 141 (97–172; *n* = 17) long, 152 (117–183; *n* = 16) wide. Anchor 81 (75–86; *n* = 21) long, with elongate robust superficial root, short deep root, straight shaft, recurved point; base 14 (12–18; *n* = 16) wide. Superficial bar 21 (13–25; *n* = 20) long, with slightly enlarged ends; deep bar rod-shaped, variably bent. Hook with straight shaft, short erect thumb, proximally tapered shank; hooklet 6 (4–7; *n* = 29) long, shank 32 (29–35; *n* = 29); domus 1/6 shank length.

REMARKS: *Hyperopletes malmbergi* sp. n. is the

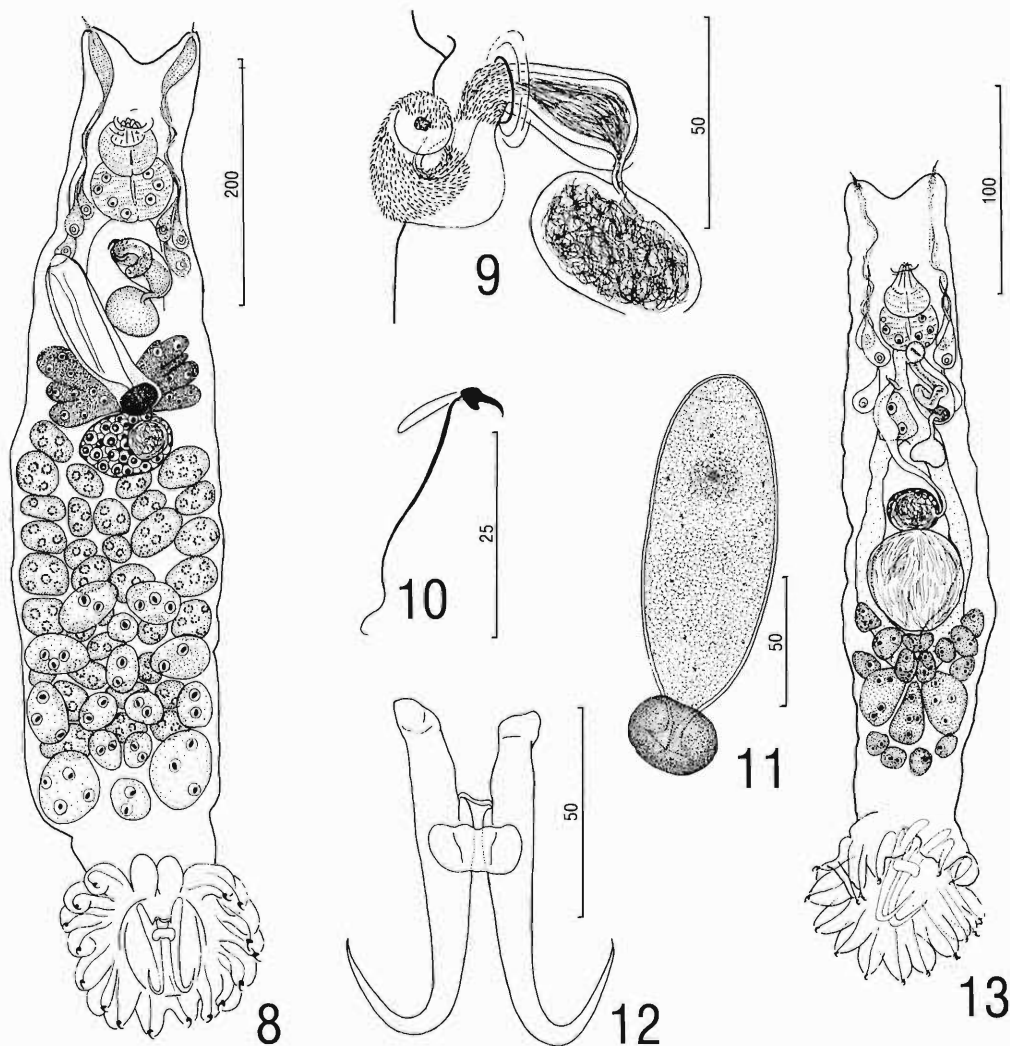


Figures 1–7. *Phanerothecium spinatus* gen. et sp. n. 1. Holotype (ventral view). 2. Terminal ducts, seminal vesicles and copulatory sac of male reproductive system. 3. Hook. 4. Ventral view of young specimen. 5. Anchor/bar complex (ventral view). 6. Egg. 7. Anchor. Figures are drawn to respective micrometer scales.

type species of the genus. It is named for Dr. Göran Malmberg, Swedish Museum of Natural History, Stockholm University, a friend, in recognition of his contributions in systematics of the Monogeneoidea.

Character Analysis

Homologous series used in the analysis follow with comments on character evolution. Numbers in parentheses preceding the definition of a



Figures 8-13. *Hyperopletes malmbergi* gen. et sp. n. 8. Holotype (ventral view). 9. Terminal portion of male reproductive system (copulatory organ is partially everted). 10. Hook. 11. Egg. 12. Anchor/bar complex (ventral view). 13. Young specimen (ventral view). Figures are drawn to respective scales.

character state refer to the coding that state received in the data matrix (Table 1); bold numbers in brackets following the definition refer to respective evolutionary changes depicted in the cladogram (Fig. 15).

A. *Eyes*. Plesiomorphy: (0) present. Apomorphy: (1) absent [1].

B. *Spike sensilla*. Plesiomorphy: (0) absent. Apomorphy: (1) present [2].

C. *Mode of reproduction*. Plesiomorphy: (0) oviparous. Apomorphy: (1) viviparous [24].

D. *Larva*. Plesiomorphy: (0) oncomiracidium ciliated. Apomorphy: (1) Larva (oncomiracidium?) unciliated [3]. Species of *Anoplodiscus*

have a ciliated oncomiracidium (Ogawa and Egusa, 1981). Although the nature of ciliation of the larvae/oncomiracidia of bothitrematids and tetraonchoids is unknown, ciliated larvae would be predicted for them based on the phylogenetic hypothesis for the Monogenoidea offered by Boeger and Kritsky (1993).

E. *Genital pore*. Plesiomorphy: (0) common. Apomorphy: (1) male and female pores separate [4].

F. *Copulatory organ*. Plesiomorphy: (0) sclerotized. Apomorphy: (1) muscular [5].

G. *Copulatory sac*. Plesiomorphy: (0) absent. Apomorphies: (1) present [15]; (2) modified into

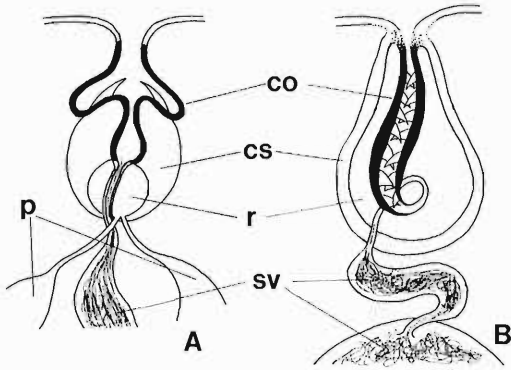


Figure 14. Schematic diagrams of terminal genitalia of viviparous (A) and oviparous (B) Gyrodactylidae showing probable homologous structures. Abbreviations: co = copulatory organ, cs = copulatory sac, p = prostate gland, r = prostatic reservoir, sv = seminal vesicles. Figure 14A is modified from Kritsky (1971).

muscular bulb [22]. In the 3 *Nothogyrodactylus* species, Kritsky and Boeger (1991) describe the copulatory sac as poorly defined, although their drawings suggest that the extrusive copulatory organ does not lie within a sac. Thus, we assigned the plesiomorphic state to *N. clavatus*, *N. plaesiophallus*, and *N. amazonicus*.

The "cirral bulb" of viviparous Gyrodactylidae apparently represents a modified copulatory sac. In these taxa, the bulb is muscular and has probably developed from the muscular wall of a plesiomorphic sac similar to that of *Ooegyrodactylus farlowellae* (see fig. 28 in Kritsky and Boeger, 1991). The prostatic reservoir in viviparous taxa is enclosed within the bulb (Kritsky, 1971) and is probably homologous to the lumen of the sac containing prostatic secretions in species of *Phanerothecium*. The copulatory organ

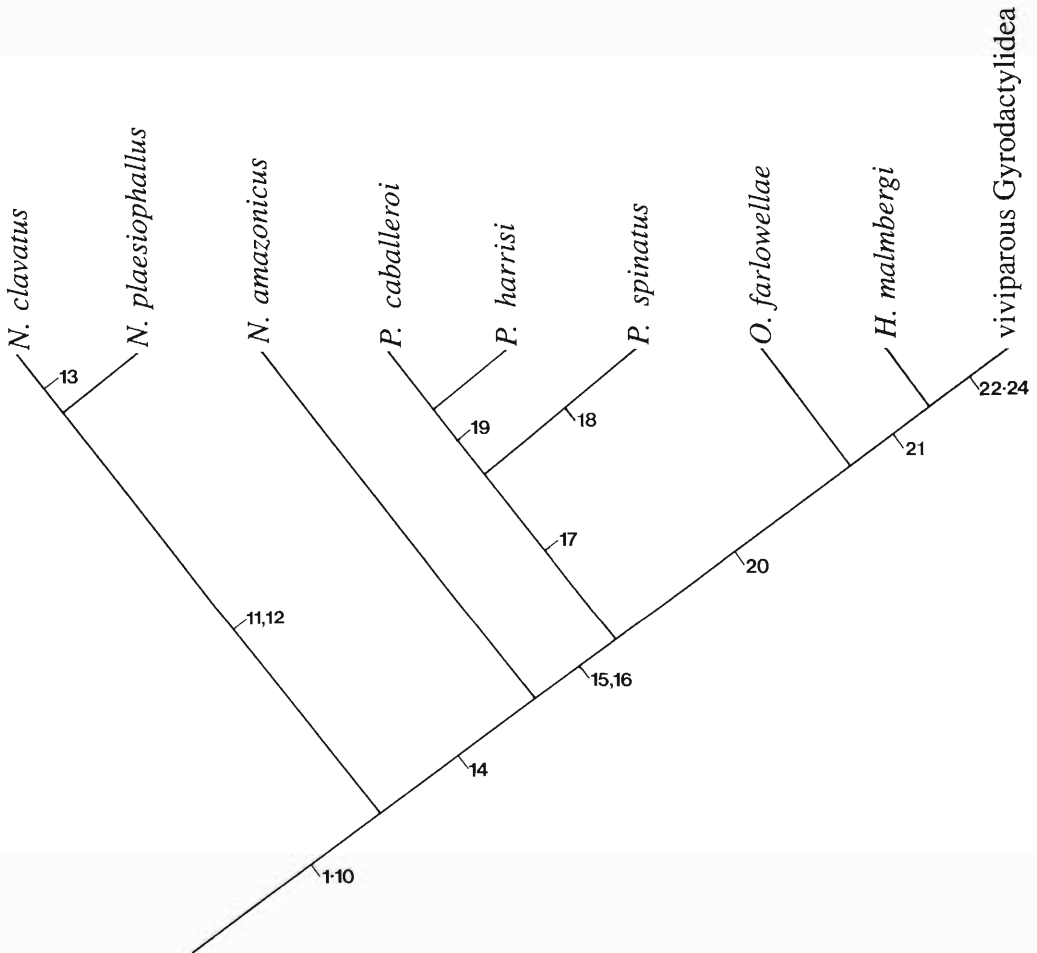


Figure 15. Cladogram depicting the evolutionary relationships within the Gyrodactylidae (sensu nobis). Slashes with numbers refer to postulated evolutionary changes in character states indicated in the text.

(the armed epithelium) of viviparous gyroductylids is eversible and lies on the superficial surface of the bulb (Fig. 14).

H. *Spines of copulatory organ*. Plesiomorphy: (0) absent. Apomorphy: (1) present [18, 21]. The analysis indicates that not all copulatory spines present in ingroup taxa are homologous. The spines of *Hyperopletes malmbergi* appear to be homologous to those of the viviparous Gyroductylidae, whereas the larger spines of *Phanerothecium spinatus* apparently evolved independently. Although we assign a (9) to the hypothetical ancestor for this series, because presence or absence of spines on the copulatory organ may be related to the type of copulatory organ present (see Series F), the analysis does not exclude the possibility that absence of spines is symplesiomorphic for the ingroup taxa.

I. *Accessory piece*. Plesiomorphy: (0) present. Apomorphy: (1) absent [16]. Although no evidence to support homology other than location exists, the accessory sclerites associated with the copulatory organ of *Nothogyrodactylus* species are considered to be homologous to the accessory piece of other Polyonchoinea based on Hennig's (1966) auxiliary principle of presumed homology. If these structures are shown not to be homologous to the accessory piece, "absence (loss) of an accessory piece" would represent another synapomorphy for the Gyroductylidae (sensu nobis) and development of the accessory sclerites as new structures would provide a synapomorphy for *Nothogyrodactylus*.

J. *Distribution of vitellaria*. Plesiomorphy: (0) follicles post- and pregermarial. Apomorphies: (1) vitellaria primarily postgermarial, with a few sinistral pregermarial follicles present (dextral pregermarial follicles absent) [14]; (2) vitelline follicles postgermarial only [20]. The morphology of the vitelline collecting ducts corresponds to the distribution of the follicles. In ventral view, species with the plesiomorphic state depict H-shaped collecting ducts, whereas those with apomorphic states have reversed h-shaped and inverted U-shaped ducts, respectively.

K. *Mehlis' gland*. Plesiomorphy: (0) comprised of small unicellular glands. Apomorphies: (1) follicular, forming a large mass of cells [6]; (2) absent [23]. In a study of the ultrastructure of the reproductive system of *Gyrodactylus*, Kritsky (1971) reported the absence of the Mehlis' gland. This absence is apparently derived and linked to development of viviparity and/or absence of an egg shell surrounding the ovum.

L. *Vagina*. Plesiomorphy: (0) present. Apomorphy: (1) absent [7].

M. *Egg cap*. Plesiomorphy: (0) absent. Apomorphy: (1) present [8]. An amorphous cap is present on the egg filament of oviparous ingroup species (egg morphology is unknown for *Phanerothecium caballeroi*). This cap is composed of a sticky substance that allows fixation of the egg or egg cluster to surfaces when laid by the parent (Kritsky and Boeger, 1991).

N. *Maximum number of eggs (or embryos) in utero*. Plesiomorphy: (0) one. Apomorphy: (1) more than one [17]. The apomorphic state does not include the condition of multiple in utero generations of embryos that may occur in viviparous forms. The viviparous taxa did not develop multiple egg production, although in utero generational development may represent another strategy for increased reproductive potential.

O. *Keel on hook shank*. Plesiomorphy: (0) absent. Apomorphy: (1) present [12, 19].

P. *Deep bar associated with deep roots of anchors*. Plesiomorphy: (0) absent. Apomorphy: (1) present [9].

Q. *Cup-like accessory sclerite*. Plesiomorphy: (0) absent. Apomorphy: (1) present [13].

R. *Sheath-like accessory sclerite*. Plesiomorphy: (0) absent. Apomorphy: (1) present [11].

S. *Hook-like accessory sclerite*. Plesiomorphy: (0) absent. Apomorphy: (1) present [10].

Series Q, R, and S indicate the presumed homology and transformation of the various accessory sclerites associated with the copulatory organs of species of *Nothogyrodactylus*. In the matrix, we assign a (0) to the hypothetical ancestor for each series because of our presumption of homology of the accessory piece of the outgroups and other Polyonchoinea and the accessory sclerites present in members of this genus (see Series I).

We initially considered the number and position of seminal vesicles as potentially useful characters for the analysis. Two seminal vesicles appear to be symplesiomorphic for the Gyroductylidae (sensu nobis). In outgroup species, *Pavlovskioides antarcticus* Bychowsky, Gusev, and Nagibina, 1965, and *P. trematomi* Dillon and Hargis, 1968 (both Tetraonchoiidae), 2 seminal vesicles are depicted by Bychowsky et al. (1965) and Dillon and Hargis (1968), respectively. Although Kritsky (1971) reported a single seminal vesicle (external) in *Gyrodactylus*, this homologous series did not provide information on phylogeny of the ingroup. Loss of the distal

seminal vesicle appears to have occurred secondarily within the Gyrodactylidae (sensu stricto), because species of *Accessorius* Jara, An, and Cone, 1991, apparently members of 1 of the first evolutionary lines to diverge within the viviparous taxon, clearly possess both proximal and distal vesicles (see fig. 1 in Jara et al., 1991). Therefore, number of seminal vesicles is a constant character that did not provide information on the evolutionary history of the ingroup taxa; consequently, this series was not used in the analysis.

Hyperopletes malmbergi is the only known species within the ingroup with the distal seminal vesicle within the copulatory sac. As such, this feature is an autapomorphy and is also not used in the analysis.

Lastly, a pharynx composed of 2 tandem sub-hemispherical bulbs was initially considered a synapomorphy for the Gyrodactylidae (sensu nobis). Members of the Tetraonchoididae and Bothitrematidae possess a pharynx comprised of a single bulb (Bychowsky et al., 1965; Dillon and Hargis, 1968), and previous reports on species of Anoplodiscidae indicated a single bulbed pharynx (see Ogawa and Egusa, 1981). We examined specimens of an unidentified species of *Anoplodiscus* from *Pagrus pagrus* (Linnaeus) collected off the coasts of Uruguay and Argentina; these specimens clearly show a pharynx comprised of 2 bulbs, suggesting that this feature is symplesiomorphic for the ingroup. Because subsequent evolution of this feature has not occurred in any of the ingroup taxa, this character was not utilized in the analysis.

Phylogenetic Analysis

The cladogram (CI = 91.7%) depicting the phylogenetic relationships of the oviparous gyrodactylids and the ancestor of the viviparous species of Gyrodactylidae is presented in Figure 15. Elimination of the homologous series that only indicate monophyly of the ingroup (Series A, B, D, E, F, L, M, P, and S) results in the same cladogram with a consistency index of 86.7%. The analysis failed to identify synapomorphies for a taxon containing just the oviparous species of the ingroup, and sister-group relationships of these taxa suggest that the Ooegyrodactylidae, as diagnosed by Harris (1983), is paraphyletic. All oviparous genera received phylogenetic support except *Nothogyrodactylus* Kritsky and Boeger, 1991, which is paraphyletic.

As a result of the analysis, the Ooegyrodac-

tylidae Harris, 1983, is rejected as a junior synonym of the Gyrodactylidae. The following genera of oviparous gyrodactylideans, and their species, are transferred to or placed within the Gyrodactylidae along with all viviparous taxa: *Hyperopletes* gen. n. (with *H. malmbergi* sp. n.); *Nothogyrodactylus* Kritsky and Boeger, 1991 (with *N. clavatus* Kritsky and Boeger, 1991, *N. plaesiophallus* Kritsky and Boeger, 1991, and *N. amazonicus* Kritsky and Boeger, 1991); *Ooegyrodactylus* Harris, 1983 (with *O. farlowellae* Harris, 1983); and *Phanerothecium* Kritsky and Thatcher, 1977 (with *P. caballeroi* Kritsky and Thatcher, 1977, *P. harrisi* Kritsky and Boeger, 1991, *P. spinatus* sp. n., and *P. sp.* [= *P. caballeroi* forma major of Kritsky and Thatcher, 1977]). Although paraphyletic, *Nothogyrodactylus* is retained for *N. amazonicus*, *N. clavatus*, and *N. plaesiophallus* until additional evidence for or against homology of the accessory piece (of outgroups) and accessory sclerites (of *Nothogyrodactylus* species) is obtained.

Discussion

Prior to Harris' (1983) discovery of the oviparous *Ooegyrodactylus farlowellae* on a South American catfish, the taxon containing the viviparous monogenoideans (the Gyrodactylidae sensu stricto) enjoyed a relatively stable acceptance and embodiment based on unique synapomorphic features. Among the most important of these characters was the viviparous mode of reproduction, which students have previously recognized as having developed only once in the evolutionary history of the Monogenoidea. However, these highly derived features without known intermediates in character evolution precluded consensus on origins, phylogenetic relationships, and classification of the taxon within the Monogenoidea (see Bychowsky, 1937, 1957; Price, 1937a, b; Yamaguti, 1963; Llewellyn, 1965; Lebedev, 1988; Malmberg, 1990). The recognition of oviparity in a gyrodactylid-like monogenoidean by Harris (1983) represented a milestone in determining sister-group relationships for the taxon (Boeger and Kritsky, 1993).

Apparently unwilling to challenge the long-standing diagnostic features of the Gyrodactylidae (sensu stricto), Harris (1983) proposed the Ooegyrodactylidae for the oviparous forms. Kritsky and Boeger (1991) provisionally accepted the family but indicated that it had a high probability for being paraphyletic because features used to establish the family "either repre-

sent symplesiomorphies of the Gyrodactylidae (sensu stricto) (misspelled 'Gyrodactylidea' in Kritsky and Boeger, 1991) or are primitive characters shared by the Gyrodactylidea and its sister taxon, the Dactylogyridea . . . (parentheses, ours) p. 14." Our discoveries of additional genera and species of oviparous gyrodactylid-like forms, including *Nothogyrodactylus* Kritsky and Boeger, 1991, allowed the present phylogenetic analysis to test monophyly of the Ooegyrodactylidae. This analysis shows the family to be paraphyletic and supports its rejection and the transfer of all included genera and species to the Gyrodactylidae. Thus, viviparity developed secondarily within the Gyrodactylidae and can no longer be used as a definitive diagnostic feature of the family. However, we were able to identify several synapomorphies supporting the new configuration of the Gyrodactylidae including (a) a muscular copulatory organ (see Series F), (b) spike sensilla in the head organs (Series B), (c) an unciliated larva (oncomiracidium absent) (Series D), (d) a deep bar associated with the deep roots of the anchor pair (Series P), (e) separate genital pores (Series E), (f) a massive Mehlis' gland (Series K), (g) an amorphous cap on the egg filament (Series M), and absence of (h) a vagina (Series L) and (i) eyes in the larva and adult (Series A).

The analysis also suggests that *Nothogyrodactylus* is paraphyletic. Kritsky and Boeger (1991) based the genus primarily on presence of 1 or more accessory sclerites associated with the extrusive copulatory organ. In the present analysis, the accessory sclerites are assumed to be homologous to the accessory piece of outgroup taxa grounded on Hennig's auxiliary principle on homology. As a result of this assumption, presence of accessory sclerites is symplesiomorphic and does not lend support to presumed congeneric status of *N. clavatus*, *N. amazonicus*, and *N. plae-siophallus*. Although no synapomorphies were identified for the group of 3 species, *N. amazonicus* is provisionally retained in the genus pending further study of character evolution within this homologous series.

Based on their respective positions within the cladogram, all other oviparous genera received evolutionary support through the analysis. Species of *Phanerothecium* share a single synapomorphy, presence of more than 1 egg in utero (Series N). *Hyperopletes* is the only species with the distal seminal vesicle enclosed within the copulatory sac (see the character analysis), and

Ooegyrodactylus has unique features associated with the morphology of the egg, egg filament, copulatory sac, and wall of the distal seminal vesicle (see Kritsky and Boeger, 1991). However, conclusive phylogenetic support for *Hyperopletes* and *Ooegyrodactylus*, both of which are monotypic, will depend on discovery of other congeneric species.

The development of viviparity in the Gyrodactylidae apparently has had profound effect on several characteristics related to structures associated with the oviparous mode of reproduction. Apparently unlike all oviparous forms, the eggs of viviparous gyrodactylids lack a shell and yolk (Kritsky, 1971). While controversy exists concerning the function of their products, absence of the Mehlis' gland and presence of apparently nonfunctional vitellaria in viviparous gyrodactylids may be related to the egg structure and/or modified mechanisms to meet embryonic nutritional needs. The phylogeny proposed herein suggests that these and similar morphological and functional characters were lost or modified in the ancestor of the viviparous Gyrodactylidae.

Some features associated with reproductive strategy, particularly those associated with parental care of offspring, appear to have begun development early in the evolutionary history of the Gyrodactylidae (sensu nobis). In general, eggs of other polyonchoineans (including the gyrodactylidean taxa used as outgroups) are maintained in utero for a short time before being released into the environment where they embryonate and eclose producing the free-swimming, ciliated oncomiracidium that actively searches for an adequate definitive host. Among oviparous Gyrodactylidae, accentuated parental care of the young has already developed. The eggs are stored in utero, where embryonation occurs (Harris, 1983; Kritsky and Boeger, 1991). The embryonated eggs are then deposited on the skin or bony plates of the host where they are secured by the sticky egg caps. Hatching occurs comparatively quickly, producing young unciliated individuals with a male reproductive system at or near maturity. Kritsky and Boeger (1991) found that eggs hatched within 6 days after expulsion in *Phanerothecium harrisi*, and Harris (1983) indicated that the male system develops rapidly and is functional about 7 days posthatching in *Ooegyrodactylus farlowellae*. Parental care of the eggs, use of the egg cap for attachment of the eggs to the skin or gills of the

host, and rapid posthatching development increase probability of survival for the young. However, the highest level of parental care for the offsprings is seen among the viviparous Gyrodactylidae, where in utero development is prolonged until the young individual approaches adult form.

Acknowledgments

The authors wish to thank the following individuals for support of this study: Rogério Nunes da Mota assisted during collecting efforts in Rio de Janeiro; Michel Jégu (Instituto Nacional de Pesquisas da Amazônia) and Heraldo A. Britsky (Museu de Zoologia da Universidade de São Paulo) provided host identifications; Flávio Popazoglo helped with laboratory work; and Sherm Hendrix allowed us to examine specimens of *Bothitrema* in his collection. The Fundação de Amparo a Pesquisas do Estado do Rio de Janeiro (Proc. E-29/170.033/90) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (Proc. 406184/90-9 and 500711/90-9), Brazil, provided funding for this project.

Literature Cited

- Boeger, W. A., and D. C. Kritsky. 1993. Revision and phylogeny of the Monogenoidea Bychowsky, 1937. *Systematic Parasitology* 26:1–32.
- Bychowsky, B. E. 1937. [Ontogenesis and phylogenetic interrelationships of parasitic flatworms.] *Izvestiya Akademii Nauk SSSR, Ser. Biologiya*, 4, 1353–1383. (In Russian: English translation, 1981, Virginia Institute of Marine Science, Gloucester Point, Virginia. Translation Series no. 26.)
- . 1957. [Monogenetic Trematodes. Their Systematics and Phylogeny.] *Izdatel'stvo Akademii Nauk SSSR, Moscow–Leningrad*. 509 pp. (In Russian: English translation, 1961, American Institute of Biological Sciences, Washington, D.C. 627 pp.)
- , A. V. Gusev, and L. F. Nagibina. 1965. [Monogenetic trematodes of the family Tetraonchoidea Bychowsky, 1951.] *Trudy Zoologicheskogo Instituta Akademii Nauk SSSR* 35:140–166. *Izdatel'stvo "NAUKA," Moscow–Leningrad*. (In Russian: English translation, 1967, Virginia Institute of Marine Science, Gloucester Point, Virginia. Translation Series No. 17.)
- Dillon, W. A., and W. J. Hargis. 1968. Helminth parasites of Antarctic vertebrates. Part III. Monogenetic trematodes from Antarctic fishes: The superfamily Tetraonchoidea Yamaguti, 1963. Pages 101–112 in W. L. Schmitt and G. A. Llano, eds. *Antarctic Research Series. Vol. 11, Biology of the Antarctic Seas III*. American Geophysical Union, Washington D.C.
- Harris, P. D. 1983. The morphology and life cycle of the oviparous *Oögyrodactylus farlowellae* gen. et sp. n. (Monogenea, Gyrodactylidae). *Parasitology* 87:405–420.
- Hennig, W. 1966. *Phylogenetic Systematics*. University of Illinois Press, Urbana. 263 pp.
- Jara, C., L. An, and D. Cone. 1991. *Accessorius peruvensis* gen. et sp. n. (Monogenea: Gyrodactylidae) from *Lebiasina bimaculata* (Characidae) in Peru. *Journal of the Helminthological Society of Washington* 58:164–166.
- Kritsky, D. C. 1971. Studies on the fine structure of the monogenetic trematode *Gyrodactylus eucaliae* Ikezaki and Hoffman, 1957. Doctoral Thesis, University of Illinois, Urbana. 530 pp.
- , and W. A. Boeger. 1991. Neotropical Monogenea. 16. New species of oviparous Gyrodactylidae with proposal of *Nothogyrodactylus* gen. n. (Ooggyrodactylidae). *Journal of the Helminthological Society of Washington* 58:7–15.
- , and J. D. Mizelle. 1968. Studies on monogenetic trematodes. XXXV. Some new and previously described North American species of *Gyrodactylus*. *American Midland Naturalist* 79:205–215.
- , and V. E. Thatcher. 1977. *Phanerothecium* gen. nov. and *Fundulotrema* gen. nov. Two new genera of viviparous Monogenoidea (Gyrodactylidae), with a description of *P. caballeroi* sp. n. and a key to the subfamilies and genera of the family. *Exerta parasitologica en memoria del doctor Eduardo Caballero y Caballero, Instituto de Biologie (Mexico), Publicaciones especiales* 4:53–60.
- Lebedev, B. I. 1988. Monogenea in the light of new evidence and their position among platyhelminths. *Angewandte Parasitologie* 29:149–167.
- Llewellyn, J. 1965. The evolution of parasitic platyhelminths. Pages 47–78 in *Evolution of Parasites*. Third Symposium of the British Society for Parasitology, Oxford.
- Maddison, W. P., M. J. Donoghue, and D. R. Maddison. 1984. Outgroup analysis and parsimony. *Systematic Zoology* 33:83–103.
- Malmberg, G. 1990. On the ontogeny of the haptor and the evolution of the Monogenea. *Systematic Parasitology* 17:1–65.
- Mizelle, J. D., and A. R. Klucka. 1953. Studies on monogenetic trematodes. XIV. Dactylogyridae from Wisconsin fishes. *American Midland Naturalist* 49:720–733.
- Ogawa, K., and S. Egusa. 1981. The systematic position of the genus *Anoplodiscus* (Monogenea: Anoplodiscidae). *Systematic Parasitology* 2:253–260.
- Price, E. W. 1937a. North American monogenetic trematodes. I. The superfamily Gyrodactyloidea. *Journal of the Washington Academy of Sciences* 27:114–130.
- . 1937b. North American monogenetic trematodes. I. The superfamily Gyrodactyloidea (continued). *Journal of the Washington Academy of Sciences* 27:146–164.
- Putz, R. E., and G. L. Hoffman. 1963. Two new *Gyrodactylus* (Trematoda: Monogenea) from cypriinid fishes with synopsis of those found on North

- American fishes. *Journal of Parasitology* 49:559–566.
- Watrous, L. E., and Q. D. Wheeler.** 1981. The out-group comparison method of character analysis. *Systematic Zoology* 30:1–11.
- Wiley, E. O.** 1981. *Phylogenetics. The Theory and Practice of Phylogenetic Systematics.* John Wiley & Sons, New York. 439 pp.
- Yamaguti, S.** 1963. *Systema Helminthum. IV. Monogenea and Aspidocotylea.* Interscience Publishers, New York. 699 pp.